

REVIEW ARTICLE

Diagnostic methods in sepsis: the need of speed

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SUMMARY

Objective: Sepsis is a common condition encountered in hospital environments. There is no effective treatment for sepsis, and it remains an important cause of death at intensive care units. This study aimed to discuss some methods that are available in clinics, and tests that have been recently developed for the diagnosis of sepsis. **Methods:** A systematic review was performed through the analysis of the following descriptors: sepsis, diagnostic methods, biological markers, and cytokines. **Results:** The deleterious effects of sepsis are caused by an imbalance between the invasiveness of the pathogen and the ability of the host to mount an effective immune response. Consequently, the host's immune surveillance fails to eliminate the pathogen, allowing it to spread. Moreover, there is a pro-inflammatory mediator release, inappropriate activation of the coagulation and complement cascades, leading to dysfunction of multiple organs and systems. The difficulty achieve total recovery of the patient is explainable. There is an increased incidence of sepsis worldwide due to factors such as aging population, larger number of surgeries, and number of microorganisms resistant to existing antibiotics. **Conclusion:** The search for new diagnostic markers associated with increased risk of sepsis development and molecules that can be correlated to certain steps of sepsis is becoming necessary. This would allow for earlier diagnosis, facilitate patient prognosis characterization, and prediction of possible evolution of each case. All other markers are regrettably constrained to research units.

Keywords: Sepsis; methods; cytokines.

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Submitted on: 10/26/2011

Approved on: 05/12/2012

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Conflict of interest: None.

INTRODUCTION

Sepsis, considered the 10th leading cause of death in the United States (US), is a challenge for medicine today¹⁻⁵, and its incidence ranges from 76 to 100 cases per 100,000 people. Sepsis and septic shock are among the major causes of death in non-coronary intensive care units (ICU)⁶⁻⁹. In Brazil, the average mortality rate is estimated to be of approximately 29%¹⁰. In addition, sepsis is not a reportable disease. As a consequence, it is estimated that these numbers may be higher, since the cause of death might be attributed to other complications. Some studies, for example, have claimed that the mortality rate due to sepsis might vary from 30% to 60%¹¹. In an evaluation in the US, involving more than 6 million hospital records in 7 states, the average number of cases of sepsis was 751,000 per year, with a mortality rate of 28.6%¹². Alarming headlines declare that incidence and mortality rates of sepsis are increasing each year. Recently, it was reported that an average of 215,000 people a year died from sepsis between 1979 and 2000 in the US, and an increase of 9% per year of mortality rate⁶. Aging population, multidrug-resistant microorganisms, popularization of invasive techniques (such as bladder catheters, endotracheal tubes, and intravascular catheters), and increasing number of surgical procedures are among the factors that might explain the higher incidence of sepsis. The peak incidence usually occurs in individuals in the 6th decade. Thus, genetic variation, unconventional lifestyle, and gender are some other factors that may increase the risk of sepsis.

Although much is known about sepsis, the disease has shown a complex clinical and therapeutic profile. Sepsis is a syndrome characterized by a systemic inflammation that can occur in the body as a consequence of a simple infection. Sepsis is actually a counteracting 'mis-response' of the body against infecting microorganisms. This uncontrolled reaction is characterized by a biased system, in favor of pro-inflammatory, pro-coagulant, and over reactive immune inflammatory response¹³. The magnitude of the response depends on several factors, such as the virulence of the organism, host genetics, and immune status. Moreover, for the progression of the infection to occur, it is necessary that the host cannot contain or destroy the primary infection. Thus, most patients develop either sepsis, or the systemic inflammatory response syndrome (SIRS), which is a clinical pro-inflammatory response, predominantly cytokine-mediated, to a nonspecific insult of either infectious or noninfectious origin. In addition, advances in cellular and molecular biology have demonstrated that bacterial invasion or its by-products (endotoxins, lipopolysaccharide – LPS) need to interact harmfully with the host's immune system for the development of

sepsis¹⁴. Moreover, among patients who develop sepsis, only a few progress to severe sepsis and are at a higher risk of death¹⁵⁻¹⁷.

In the progress of the uncontrolled inflammatory response in sepsis, unpredictable cardiovascular phenomena occur, such as hypovolemia, peripheral vasodilation, myocardial depression, increased endothelial permeability, and hypermetabolism. Patients with sepsis according to a consensus established at the International Conference of Sepsis¹⁴ show a diversity of clinical signs. Temperatures can vary from higher than 38°C to lower than 36°C, heart rate may be above 90 beats per minute (bpm), tachypnea (FR > 20/min) or hyperventilation (pCO₂ < 32 torr) may be present, and white blood cell count may be above 12,000 or below 4,000 cells/μL^{14,18,19}.

Severe sepsis is characterized when there is association of sepsis with organ dysfunction. Septic shock, on the other hand, occurs when resuscitation maneuvers are mandatory due to hypotension or persistent changes in tissue perfusion after conservative attempts of hemodynamic homeostasis maintenance are performed. Nevertheless, the boundaries between severe sepsis, septic shock, and multiple organ dysfunctions are not clearly defined in clinical practice¹⁹⁻²¹.

Several inflammatory markers have failed to fulfill the requirements to be eligible for an early and reliable diagnostic predictor of sepsis. Studies^{17,22} have shown that the best policy is to consider a combination of markers, since the majority of the studies in animal models are not reproducible in humans. This, added to the fact that several markers are commonly found in a wide range of diseases, creates a great difficulty in relating laboratory data to the patients prognosis. Clinical laboratory diagnosis is crucial to avoiding delay in treatment²³⁻²⁵. Thus, one-quarter of patients with sepsis have inadequate treatment and worse prognosis as a consequence of a delayed diagnosis^{12,17,22}. In summary, diagnosis can be considered a two-sided process. Firstly, finding biomarkers capable of monitoring metabolic homeostasis and constantly evaluating patient severity, indicating whether there is systemic or specific organ involvement. Secondly, finding a marker related to pathogen identification, through a quick screening of the patient²⁶. A diagram depicting the diagnostic sepsis phases is presented in Figure 1. In other words, laboratory tests should aim to identify compromised systems or organs, including indicators of inflammatory response in peripheral blood (pro-inflammatory mediators and acute phase indicators) and indicators of organic disorders. Enhanced serum lactate, cytokines, colony stimulating factor, and granulocyte markers of inflammation may be early indicators of more severe conditions, such as SIRS.

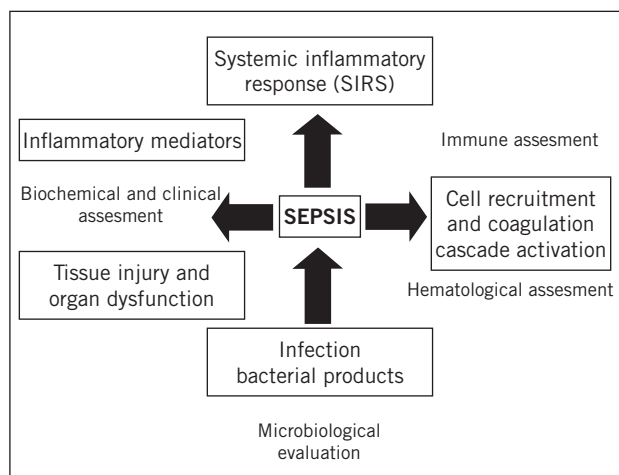


Figure 1 – Diagnostic sepsis phases.

METHODS

The data presented in this review originated from a systematic analysis of the PubMed database over the last 10 years by searching for the following terms: sepsis diagnostic, sepsis methods, biological markers, and cytokines. The authors have attempted to summarize and to reference the literature about sepsis and laboratory diagnostics.

HEMATOLOGICAL ASSESSMENT

Blood count analysis and peripheral blood smear tests are usually the first results to be obtained and can provide important information for the clinical management of patients. Frequent leukocytosis is noticeable in patient with sepsis. However, leukopenia or pancytopenia may also be found²⁷. Neutrophilia with a left shift and the presence of immature granulocytes with toxic granulations can be found. The latter, when present in large numbers, is a marker of severity of infection.

Inflammatory mediators cause increased vascular permeability and immune cells chemotaxis to the site²⁸⁻³³. In this process, neutrophils move out of the capillaries, enter the tissues, releasing proteolytic enzymes and reactive oxygen species³³⁻³⁵. Platelets are then attracted to and stick to the damaged endothelium. Platelets and leukocytes occlude the microvasculature leading to a decrease in the blood flow. Following, there is a need to increase oxygen offered to tissues, but this is impossible due to vascular changes.

In addition, there is activation of the coagulation cascade secondary to the complement system, causing a reduction of anticoagulants factors¹³. In patients with microvascular coagulopathy, it is possible to find different degrees of thrombocytopenia and microangiopathy, with or without the presence of erythroblasts, and schizocytes³⁶. Thrombocytopenia is an independent prognostic marker of mortality in sepsis and should be carefully investigated. The course of sepsis may be the result of drug use,

post-transfusion purpura, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, or heparin-induced thrombocytopenia³⁷. Thus, these candidates for diagnostic markers should be intensively investigated in the near future.

BIOCHEMICAL ASSESSMENT

In sepsis there is a great variation of the patient's electrolytes levels and elevated levels of liver enzymes due to hypoxia. There is also a pronounced hyperglycemia with a hyper- or hypocoagulable state, and metabolic acidosis with respiratory compensation and increased anion gap due to lactic acid production are commonly found³⁸.

The use of biochemical diagnostic markers is vital to determine the prognosis of the patient. The use of gas analysis and other tests such as lactate, albumin, C-reactive protein (PCR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and hemopexin levels are important not only to indicate patient status, but also to give a good picture of the changes in blood homeostasis^{20,39-41}. Gas analysis also provides important information regarding the patient's need for fluid replacement, since the presence of elevated PCR, ALT, and AST is relatively common in sepsis. This may be due to liver injury, post-ischemic condition, and it might also indicate drug toxicity, or be a synonym of direct aggression of the hepatocytes with mitochondrial dysfunction²⁰. Lactate levels, for which tests are available in most hospitals and are relatively low cost, appear to be associated with increased mortality risk in sepsis⁴².

Albumin is an independent diagnostic marker of severity and mortality in sepsis. During inflammation, there is a leakage of serum albumin to the interstitium and albuminuria in varying degrees. The claimed replacement of exogenous albumin remains controversial in terms of mortality reduction⁴³. The albumin role is better established in patients with liver dysfunction, ascites, and acute lung injury⁴³. Natriuretic peptides (NP) are released by atrial distension and play important role in regulating blood volume, and are considered markers of heart failure. In patients with septic shock, increased levels of NP are associated with higher mortality by myocardial-depression. Recent studies with ICU patients demonstrated that NP levels were significantly higher in survivors than in non-survivors, therefore they are a potential indicator of positive prognosis in sepsis⁴⁴⁻⁴⁸.

Blood lactate levels can also be useful in evaluating severe sepsis⁴⁹⁻⁵⁰. Serum lactate is a good indicator of the presence of hypoxic tissue during septic shock, since its production takes place during anaerobic metabolism⁵¹. Research conducted by Arnold et al.⁵¹ and Nguyen et al.⁵² provided results suggesting that the clearance lactate rate measure is important in order to identify patients who

better respond to treatment and have a more favorable prognosis. In addition, serial measurements of lactate levels in septic patients are more appropriate to assess disease progression rather than a single measurement⁵¹. The determination of plasma procalcitonin (PCT) can also be valuable in early diagnosis of patients with severe sepsis⁵³. PCT is the precursor of calcitonin, and higher levels are associated with the development of severe sepsis. Commonly, there is an elevation of PCT levels 4 h after the onset of symptoms, peaking between 8 h and 24 h⁵⁴. There is evidence that PCT levels are higher in infections caused by Gram-negative than those caused by Gram-positive bacteria⁵⁵ and it seems to be more specific than PCR^{18,56}.

Ferritin, serum iron binding capacity, and transferrin are tests that must be interpreted with caution in septic patients, since all may be changed due to the presence of high levels of hepcidin⁵⁷. Ferritin is an acute phase protein that is usually elevated in sepsis and reflects the status of iron stores. The authors of the present study proposed that the level of soluble transferrin receptor should be a good assessment of iron store levels, but even this test has its accuracy reduced in cases of sepsis. The interpretation of these tests should be performed in conjunction with the level of hemoglobin and reticulocytes. Another interesting test reported is insulin sensitivity test⁵⁸⁻⁶¹. According to Lin et al., glycemic sensitivity reflects the physiological state of the patient together with temperature, heart rate, breathing score, and blood pressure. Careful studies on the changes of these rates would allow physicians to elaborate a mathematical model to predict the onset of sepsis and the need for antibiotics^{58,62}.

MICROBIOLOGICAL EVALUATION

Despite numerous advances in the diagnosis of sepsis, the microbiological evaluation has not yet lost its importance, since it is paramount to identify the causative agent in order to choose an adequate antibiotic therapy. Microbiological evaluation includes direct tests and blood (at least two) or other body fluid culture such as urine, cerebrospinal fluid, feces, secretions, and exudates^{62,63}. Preferably, the sample collection should be performed before the use of antimicrobial therapies. For hospitalized patients, material for culture can also be collected through other methods, including venous or arterial catheters (blood catheters), urinary catheter, tracheotomy (tracheal aspirate), and sutures or scars from recent surgeries⁶². Although blood culture is the currently adopted method, several patients with sepsis have negative results for this exam. In the ICU, the main causative agents of sepsis are *Staphylococcus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, *Acinetobacter baumannii*, and *Candida* spp.^{64,65}. The use of automated systems for monitoring

blood cultures (VITEK, ESP Culture Trek Diagnostic Systems; BacT / Alert and BACTEC the bioMérieux BD) increases the speed and improves their efficiency^{63,64}. Most systems monitor the consumption of carbon dioxide (CO₂) by colorimetric methods, generally detecting positivity after 48 hours. An important point to highlight is the way in which the sample collection is performed, in order to avoid false positives and contamination with skin flora.

IMMUNE ASSESSMENT

During an immune response, there is activation of various mechanisms of host defense against a pathogen, such as inflammation, complement and coagulation cascades, polymorfonuclear (PMN) activation, and chemoattraction to the site^{13,27}. These processes are over reactive in SIRS condition. This exacerbated activation of the inflammatory response may lead to greater cell damage, which can culminate with an impairment of the immune response. Despite the fact that only a few tests are commercially available for clinical evaluation purposes, there are several other inflammatory mediators that are screened in the experimental field. Among those markers used as mediators in sepsis there are cytokines such as tumor necrosis factor alpha (TNF)- α , interleukin (IL) -1, IL-6, IL-8, IL-10, and interferon (IFN)- γ ⁶⁶⁻⁶⁹, and the presence of bacterial products in the blood or the bacterium itself⁷⁰⁻⁷¹. IL-1 and TNF- α cytokines are the first cytokines released in an infection and stimulate cellular response. These cytokines release secondary mediators, resulting in chemotaxis and granulocyte activation^{28,33}. These, in turn, lead to another round of cytokine outbreak, which could also give a good picture of the inflammatory process if they could be assessed.

A good example of an acute mediator is IL-6, which is also a predictor of the severity and prognosis of sepsis. Nonetheless, this cytokine lacks specificity, since is likely to be present in high levels in several other inflammatory processes^{60,72-74}. For the same reason, TNF- α cannot be used for the diagnosis of sepsis. IL-10 is an anti-inflammatory cytokine and its level appears to indicate whether the patient is able to respond to an aggression. Bacteria and their by-products, such as LPS and lipoprotein binding protein (LBP), are also good inflammation markers. LBP is an acute phase protein involved in immune response mediated by endotoxin that allows the endotoxin to bind to CD14 receptor, which subsequently activates the expression of toll-like receptors (TLR)-2 and TLR-4 and triggers gene transcription⁷⁵.

Unfortunately, all these markers are common to many different inflammation types and are also found in simple infections, and thus, are not good predictors of inflammation severity. Also, it is necessary to process the sample immediately, because these proteins are labile and can easily

change or be degraded. Once again, as previously mentioned, it is imperative to find a “pathognomonic” marker for sepsis that can routinely be used for clinical diagnosis.

MOLECULAR DIAGNOSIS

The use of molecular biology techniques to diagnose new cases of sepsis is necessary. This has been strongly encouraged by the requirement of smaller samples, capable of providing reliable results and earlier diagnosis. These techniques can detect the presence of LPS in the blood, expression of High-Mobility Group Box (HMGB) -1 or even identify bacterial DNA⁷⁶. These tests, however, are not 100% accurate, but they do strongly indicate the presence of sepsis. Detection of bacterial DNA fragments by real-time polymerase chain reaction (RT-PCR) in blood samples, or 16S rRNA fragments of Gram-positive and Gram-negative bacteria and *Candida* in the 18S rRNA might be very promising to help early detection of sepsis, since they have shown a high degree of specificity and sensitivity. The main disadvantages of these techniques are the high costs, the lack of standardization, and the need for skilled personnel to perform them.

The ideal test should be precise, affordable, reproducible, fast, and show high specificity and sensibility, being able to accurately evaluate the patient during different stages of the condition. Until now, none of the tests fulfilled these conditions.

SCORING PREDISPOSITION TO SEPSIS

The criteria for diagnosis of sepsis was established in 1991, and revised only at the International Sepsis Definitions Conference in 2001³⁷. The risk and individual symptoms during sepsis were defined as PIRO: predisposition to infection and response to organ dysfunction^{37,77}. This score is important to establish a correct and personalized treatment implementation in sepsis. The PIRO score uses several indicators such as prior co-morbidities, gender, age, culture and characterization of the sensitivity of the microorganism, SIRS, manifestations of sepsis, shock, PCR, and failure rate of organ dysfunction³⁷. Another widely used diagnostic criterion is the age acute physiology and chronic health examination (APACHE)⁷⁸⁻⁷⁹, but it is more restricted and sometimes fails to differentiate sepsis from SIRS. In addition, the information obtained from the monitoring of indicators of severity (sepsis-related organ failure assessment – SOFA) may be more appropriate in many cases⁸⁰.

CONCLUSION

Rapid diagnosis is essential in the case of sepsis. Laboratory findings are important and represent a two-sided process. The first side is responsible for monitoring changes in metabolic homeostasis and patient evaluation; indicating

severity of the disease and whether there is involvement of specific organs or entire systems. The second refers to pathogen identification through a microbiological screening of the patient.

Several indicators might be used for this purpose: pro-inflammatory mediators, acute phase indicators, and pathogen metabolites. Lactate levels, serum cytokines, presence of colony stimulating factors, and plasma nitric oxide levels may be early indicators of SIRS, but remain restricted to research units.

An ideal test should allow for a fast and precise diagnosis, be reproducible, affordable, and have high sensitivity and specificity. Despite several candidates such as blood culture, serum lactate, and PCT levels, a combination of tests is still compulsory for the diagnosis of sepsis. In a field where speed and accuracy are needed, a gold standard test for sepsis is still searched for. Because health is so precious, knowledge must rise to meet current needs.

ACKNOWLEDGEMENTS

The authors apologize to the many researchers whose work they have not been able to discuss in this limited review. Joilson Oliveira Martins is supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Projeto Universal), and Pró-reitoria de Pesquisa da Universidade de São Paulo (Projeto I and Novos Docentes), Brazil.

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